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Seroprevalence of COVID-19 IgG Antibodies among Health Care Workers of Pakistan

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Contributor ship statement:

HB: Paper write-up, data collection and analysis, literature review.

ORC: Original concept, study design, supervision.

MDK: Proofreading, approval, discussion.

ASC: Proof reading and approval

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JK: Data entry and analysis

Abstract:

Objective: In this study we aimed to find the seroprevalence of health care workers (HCW) of Pakistan involved in the treatment and care of COVID-19 patients.

Setting: This was a cross sectional study and total of 15000 HCW, involved in providing services and care to the COVID-19 patients were randomly selected from all over Pakistan.

Participants: Informed consent was taken from all participants and were included according to inclusion and exclusion criteria. All testing was done on serum samples for the qualitative detection of SARS-CoV-2 IgG antibodies using Abbott Chemiluminescent microparticle immunoassay. An index of 1.4 was used as a cut off to mark reactive and non-reactive cases. SPSS version 23.0 was used for data analysis.

Outcome: Immune status of the study population depicting seroprevalence among HCW

Results:

Out of all the candidates, majority of the HCW were males (61.9%) and were doctors (62.4%). The mean age of participants was 32.8 years (SD 8.7) and majority were asymptomatic (51.8%). In this study, 33% of the HCW were reactive for SARS-CoV-2 IgG antibody. Around 44% of the reactive cases were asymptomatic. Factors which showed significant association with the presence of antibodies were professional category, type pf personal protective equipment available and living arrangement ($p<0.05$).

Conclusion:

Our study showed a high seropositivity of health care workers dealing with COVID-19 patients in Pakistan revealing significant association with professional category, nature of work place and precautions taken while performing duties.

Key words: Serosurveillance, Seropositivity, health care workers, COVID-19, SARS-CoV-2, IgG antibodies

Summary:

- Subclinical or asymptomatic cases can play a major role in SARS-CoV-2 transmission while remaining undiscovered in the community
- Health care workers (HCW) fall in the category of population which is most exposed to SARS-CoV-2 infection
- Serosurveillance of HCW is an important indicator of COVID-19 spread
- In this study we aimed to find the seroprevalence of health care workers of Pakistan involved in the management and care of COVID-19 patients
- 33% of the health care workers were reactive for SARS-CoV-2 IgG antibody and 44% of the reactive cases were asymptomatic
- Our study revealed that there is a significant association between use of personal protective equipment, professional category, place of duty and seropositivity
- This study can help health care policy makers of Pakistan to know to extent of infection among the health care workers and make better strategies

Introduction:

COVID-19 (Corona Virus Disease 2019) associated with Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has emerged as a pandemic. Since the identification of initial cases of this atypical pneumonia, there has been an exponential increase in the number of cases worldwide.[1]Some of the few causes of this exponential increase in number include high transmission rate especially among the asymptomatic cases, poor health care strategies and limited knowledge about the novel corona virus.[2] Subclinical or asymptomatic cases are one of the major challenges in this pandemic as it is presumed that these cases can play a major role in infection spread while remaining undiscovered in the community.

As of August 22, 2020, confirmed cases of COVID-19 in Pakistan are 292,150 with 731 critical cases and 275,317 recoveries.[3] The correct estimate of the spread of COVID-19 in Pakistan is still not clear, posing a huge challenge to health care agencies of Pakistan. Most of the mildly symptomatic, asymptomatic cases and the cases belonging to areas of limited resources who are not tested via government health care agencies can easily be missed and the actual number of the affected individuals remains unidentified. The risk of COVID-19 among health care workers (HCW) of Pakistan is still not clear. Studies have revealed that 9% of the individuals in Italy and 26% of the individuals in Spain who were tested positive for COVID-19 via Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) in prevalence studies were health care workers. [4-5] In accordance with other coronaviruses, health care workers associated transmission of COVID 19 appears to play a major role in infection spread. One prevalence study suggested that more than 40% of the diagnosed cases of COVID 19 were assumed to have been acquired from health care workers.[6]

Determining the rate of seropositivity is important as majority of the SARS-CoV-2 infected cases remain asymptomatic.[7] A reasonable degree of immunity is expected among the survivors of COVID-19.[8] According to one estimate, herd immunity can be ensured if around 60% to 80% of the population develops immunity against SARS-CoV-2. RT-PCR is used for COVID-19 diagnosis but the diagnostic tests cannot be employed to assess seroprevalence. Seroconversion is determined by analyzing antibody status of the population. [9-10] Serosurveillance of HCW is an important indicator of COVID-19 spread as they fall in the category of population which is most exposed to SARS-CoV-2 infection.

In this study we aimed to find the seroprevalence of health care workers of Pakistan involved in the management and care of COVID-19 patients. This study will help to identify the high-risk subgroups of health care workers, high risk departments and different factors associated with the risk of SARS-CoV-2 infection.

Methodology:

Subject selection:

This was a cross sectional study approved by the "**Institutional Review Board of Chughtai Institute of Pathology",** Lahore, Pakistan under approval number CIP/IRB/1038. A total of 15000 HCW, both male and females were randomly selected for this study from all over Pakistan. Only the HCW involved in providing services and care to the COVID-19 patients were involved in this study. The HCW with no interaction with COVID-19 patients (either direct contact or dealing with patient samples) or the workers

who were not present on duty in the last one month from the date of commencement of study were excluded. Moreover, HCW who were already involved in some clinical trials of COVID-19 were not involved in the study. All participants were explained the objective of the study and the HCW showing active signs and symptoms compatible with COVID-19 were not involved in the study. After taking a written informed consent, the participants were asked to fill a questionnaire in which they had to mention their age, gender, professional category, place and nature of duty, kind of personal protective equipment provided to them, presence of sign and symptoms and living arrangement. A 3 ml random blood sample was collected from each participant to assess immunological response. Sample was collected by trained phlebotomist wearing appropriate personal protective equipment. All the samples collected were transported immediately to the laboratory where those were centrifuged at 5000g for 5 min to separate sera for analysis.

Patient and public Involvement:

HCW involved in providing services and care to the COVID-19 patients were involved in the design and conduct of this study. Discussion sessions were arranged with a group of health care workers to discuss the feasibility of the project, designing proforma, outcome measures and sample collection procedure. This discussion group considered participants' priorities and preferences while designing the study. All the participants will be informed about the results of the study after publication through phone calls and lab reports posted if desired by the study participants.

Laboratory analysis:

Analysis was done on serum samples for the qualitative detection of SARS-CoV-2 IgG antibodies using FDA (Food and Drug Administration) approved Abbott Chemiluminescent Microparticle Immunoassay on Abbott Alinity Ci. This is an EUA (Emergency Use Authorization) granted assay with a specificity of 99.6% and sensitivity of 96.7% for COVID-19 confirmed cases with more than 14 days of symptoms. An index of 1.4 was used as a cut off to mark reactive and non-reactive cases. Cases with results greater than the cut off value were marked as reactive and those with results less than the cut off were declared as non-reactive for SARS-CoV-2 IgG antibodies.

Statistical analysis:

SPSS version 23.0 was used for data analysis. Seroprevalence was calculated and associations between variables of the study were tested via Chi Square and Fisher's exact test using p value <0.05 as significant. Relative risk was expressed as odds ratio.

Results:

Baseline characteristics of the participants:

A total of 16882 HCW were randomly approached for the survey between 1st July 2020 to 20th July 2020. Of these, 1672 were excluded from the survey on the basis of exclusion criteria, 210 fulfilled the inclusion criteria but refused to participate and 15000 cases were selected with a total participation rate of 88.8%. These health care workers included doctors, nurses and paramedical staff directly involved in providing medical care to the COVID-19 patients. Out of all the cases, majority of the HCW were males (61.9%) and were doctors (62.4%) followed by paramedical staff (22%). The mean age of participants was 32.8 years ±8.7 and majority were asymptomatic (51.8%). The nature of personal protective

equipment provided to health care workers, the place of duty, living arrangements along with some other characteristics of the participating HCW are mentioned in table 1.

Tables:

Table 1: Baseline Characteristics of health care workers participating in the study

Groups	Subgroups	Total Number	Percentages
Gender	Male	9282	61.9
	Female	5718	38.1
Age (years)		Mean 32.8 ±8.7	
Category	Doctor	9362	62.4
	Nurse	2260	15.1
	Paramedics	3378	22.5
Place of duty	General Ward	6327	42.2
	OPD / Clinic	4010	26.7
	COVID-19 Quarantine center	291	1.9
	COVID-19 Isolation Ward	2820	18.8
	HDU / ICU	1445	9.6
	Emergency Unit	32	.2
	Laboratory	32	.2
	Operation Theater	43	.3
Tyvek Suits	Not Available	7784	51.9
	Available	7216	48.1
Face Shield	Not Available	9518	63.5
	Available	5482	36.5
Safety Goggles	Not Available	10299	68.7
	Available	4701	31.3
Gloves	Not Available	3849	25.7
	Available	11151	74.3
N95 Masks	Not Available	6514	43.4
	Available	8486	56.6
Surgical Masks	Not Available	4149	27.7
	Available	10851	72.3
Aymptomatic		7768	51.8
Reported COVID-19 compatible symptoms within last 2 month		6260	41.7
Experienced generalized fatigue only		972	6.5

Living arrangement	Living alone	665	4.5
	Living with family	14335	95.5

Seroprevalence of SRAS-CoV-2 IgG antibodies among the health care workers:

In this study, 33% of the health care workers were reactive for SARS-CoV-2 IgG antibody and maximum percentage of HCW (46%) showing seropositivity were between 23 to 30 years of age. Out of all the tests conducted, maximum number of participants were from Punjab (10,943) followed by Khyber Pakhtunkhwa (2606) (Figure 1) City wise seropositivity is given in Figure 2.

Around 2223 (44%) of the reactive cases were asymptomatic. The symptoms more significantly associated with seropositivity were; Low Grade fever only (OR 1.31 CI 1.16-1.48), High grade Fever with headache (OR 2.43 CI 2.16-2.73), Fever with cough and shortness of breath (OR 2.10 CI 1.91-2.31), Loss of sense of smell or taste only (OR 3.70 CI 3.29-4.17) (p <0.001). Factors which showed significant association with the presence of antibodies were professional category, place of duty, availability of protective masks, safety goggles and living arrangements (p <0.05). Relative risk of different variables expressed in terms of Odds ratio is given in table 2.

Table 2: Factor analysis of different variables associated with seropositivity

Groups	Subgroups	Non-Reactive	Reactive	pvalue	OR	(95% CI)
Gender	Male	6117	3165	<0.001	0.87	0.81-0.94
	Female	3933	1785			
Category	Doctor	6572	2790	<0.001		
	Nurse	1409	851			
	Paramedics	2069	1309			
Place of duty	General Ward	4309	2018	0.04		
	OPD / Clinic	2623	1387			
	COVID-19 Quarantine center	197	94			
	COVID-19 Isolation Ward	1902	918			
	HDU / ICU	950	495			
	Emergency Unit	21	11			

	Laboratory	16	16			
	Operation Theater	32	11			
Tyvek Suits	Not Available	5184	2600	0.27	0.963	(0.89-1.03)
	Available	4866	2350			
Face Sheild	Not Available	6331	3190	0.083	0.93	0.875-1.00
	Available	3719	1760			
Safety Goggles	Not Available	6829	3470	0.008	.904	0.84-0.97
	Available	3221	1480			
Gloves	Not Available	2592	1257	.601	1.021	0.944-1.104
	Available	7458	3693			
N95 Masks	Not Available	4280	2234	.003	.902	0.842-0.966
	Available	5770	2716			
Surgical Masks	Not Available	2812	1337	.212		
	Available	7238	3613			
Aymptomatic		5545	2223	<0.001	0.66	0.61-0.70
Reported COVID-19 compatible symptoms within last 2 month		4505	2727			
Living arrangement	Living alone	476	189	0.001		
	Living with family	9574	4761			

OR= Odds ratio, CI= Confidence interval

Discussion:

Serosurveys are essential in the management of infectious diseases to assess the immunity in a population.[11] This is the first study reporting SRS-CoV-2 IgG antibodies seroprevalence among health care workers in Pakistan who are the frontline warriors in this pandemic. Pakistan is a developing nation with limited medical resources and health care workers are not provided with all the necessary

equipment to protect themselves from this deadly virus. In this study we found a seropositivity of 33% among health care workers of Pakistan with highest seropositivity in the Punjab region of Pakistan with maximum disease burden.[3]The association of antibodies with virus neutralization, correlation of antibody titers with protection against re infection and difference in immunological response of symptomatic and asymptomatic cases is still unclear.[12] However the role of serological assays in assessing the prevalence of COVID-19 in the community is unquestionable.[13] We designed this study to investigate seroprevalence of SARS-CoV-2 IgG antibodies among the population involved in direct dealing with COVID-19 patients (either the patients or direct dealing with patient samples) with FDA approved assay having high analytical performance capable to identify IgG antibodies against N protein of the virus.[14] Given the fact that HCW are at a very high risk of COVID-19, from our findings with a seropositivity of 33% among HCW it can be assumed that Pakistani population is still far from reaching more than 60% herd immunity level that is required for community protection against the infection. [15]

To date, many studies have been conducted worldwide to unfold prevalence of SARS-CoV-2 IgG antibodies among health care workers. Screening of symptomatic health care workers has been done with the help of RT-PCR in some surveillance studies and it was found that almost 18% of the HCW were infected by SARS-CoV-2.[16-17] Another screening study on asymptomatic HCW revealed that 3% of the staff was SARS-CoV-2 positive by RT-PCR .[18] In one study, the authors speculated that frontline health care workers are more prone to be infected by the virus. Not only the health professionals who are working in the dedicated COVID-19 health facilities are expected to be exposed but also the workers who are performing duties in other specialties where SARS-CoV-2 patients are admitted for other reasons.[19] A study conducted on women admitted in labor room to give birth showed that 15% of the expecting mothers were SARS-CoV-2 positive and another study in old care home revealed 4% of the residents to be positive.[20-21] A recent seroprevalence study conducted in Denmark showed that 4% of the health care workers showed seropositivity against SARS-CoV-2 and the risk of viral infection was related to the level of exposure to the infected patients.[22] This study also revealed that seroconversion was higher in male health care workers as compared to females but the authors attributed this finding to higher frequency of critical illness in males rather than higher susceptibility of infection in male gender.[22] This finding is in accordance with our study in which male health care workers showed more frequent seroconversion as compared to females and seropositivity had an association with direct contact with the patients.

An Italian study found that 4.6% of the health care workers were seropositive, majority were men and low prevalence was found in asymptomatic cases.[23] All these findings are in accordance with our study which yielded similar findings. Another study conducted at a large Spanish hospital found a seroprevalence of 9.3% in a random sample of health care professionals and the authors concluded that HCW are at a higher risk to acquire SARS-CoV-2 infection from fellow workers or directly from the patients.[24] Our study revealed that there is a significant association between presence and absence of PPE and seropositivity. Some studies suggest that seropositivity is attributable to institutional application of proper PPE use hospitals which prepared well before time for this pandemic had a lower infection rate among their health care workers.[23] A special communication regarding risk assessment of health care workers performing duties at high risk areas suggested that lack of understanding of the disease course, improper use of PPE, stress at work, unavailability of screening tests and lack of resources are the major factors leading to high rate of infection among this cohort.[25] Our study has

some limitations like only the antibody against the N protein was assessed, subjects were not followed up to understand the duration of seropositivity and protection from getting re infected again.

Conclusion:

This is a large-scale study conducted in all provinces of Pakistan showing a high seropositivity of health care workers dealing with COVID-19 patients. The seropositivity had significant association with place of duty, professional category and availability of PPE. This study can help health care policy makers of Pakistan to know to extent of infection among the health care workers and make better strategies to protect the front-line warriors in this pandemic.

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Chughtai Lab, Lahore, Pakistan.

ETHICAL APPROVAL:
Ethical approval was obtained by **"Institutional Review Board of Chughtai Institute of Pathology"** prior to initiation of the research work under approval number

PATIENTS' CONSENT:
Informed consent was obtained from all patients to publish the data concerning this study. However, there is no chance that participants can be identified from the text or illustrations of this study.

CONFLICT OF INTEREST:
Authors declare no conflict of interest.

DECLARATION OF TRANSPARENCY:

Dr. Hijab Batool affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

Note: No additional data.

Figures:

Figure1: Total number of tests conducted in each province of Pakistan and respective seropositivity

Figure 2. Seropositivity of health care workers in different cities of Pakistan

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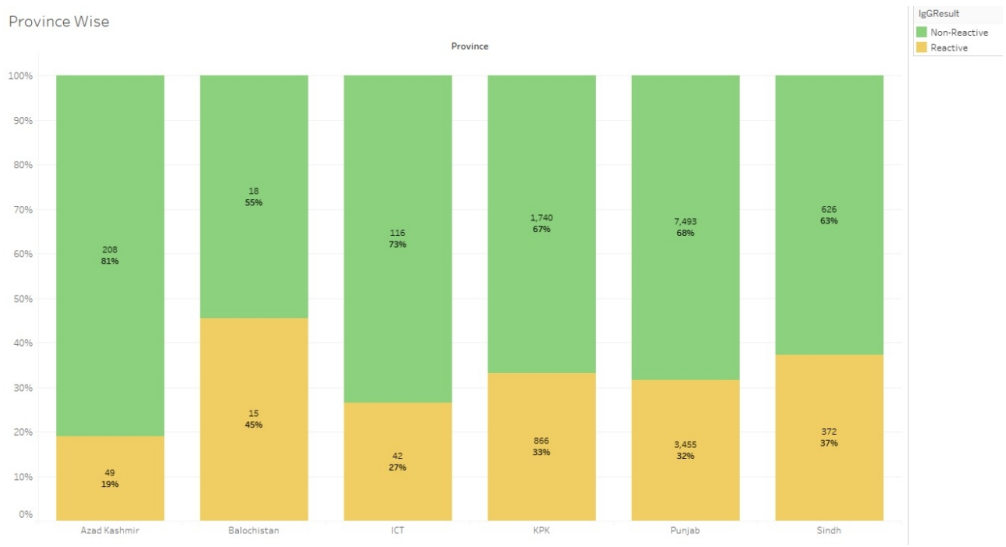

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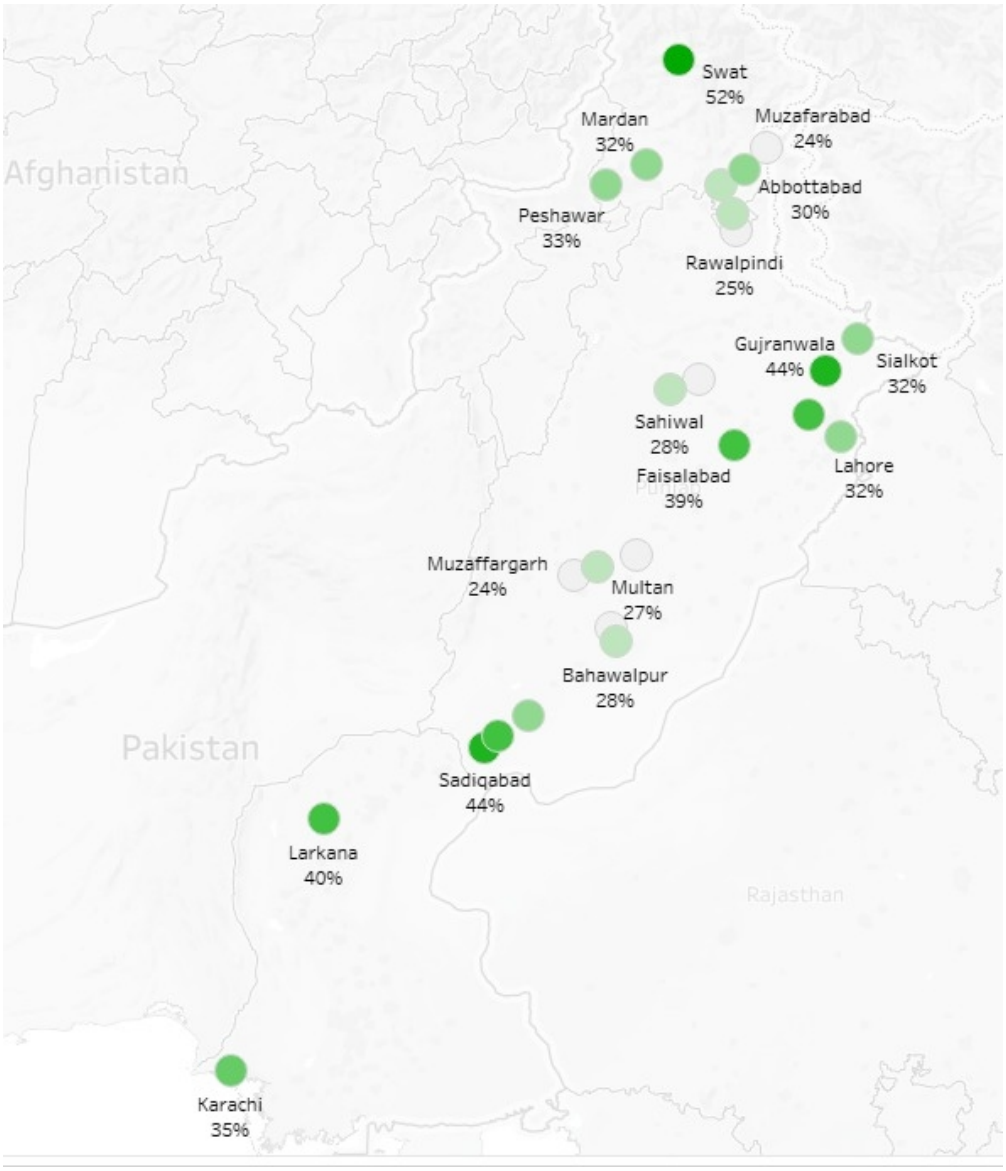

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Contributor ship statement:

HB: Paper write-up, data collection and analysis, literature review.

ORC: Original concept, study design, supervision.

MDK: Proofreading, approval, discussion.

ASC: Proof reading and approval

SA: Data collection, statistical analysis.

JK: Data entry and analysis

Abstract:

Objective: In this study we aimed to find the seroprevalence of health care workers (HCW) of Pakistan involved in the treatment and care of COVID-19 patients.

Setting: This was a cross sectional study and total of 15000 HCW, involved in providing services and care to the COVID-19 patients were randomly selected from all over Pakistan.

Participants: Informed consent was taken from all participants and were included according to inclusion and exclusion criteria. All testing was done on serum samples for the qualitative detection of SARS-CoV-2 IgG antibodies using Abbott Chemiluminescent microparticle immunoassay. An index of 1.4 was used as a cut off to mark reactive and non-reactive cases. SPSS version 23.0 was used for data analysis.

Outcome: Immune status of the study population depicting seroprevalence among HCW

Results:

Out of all the candidates, majority of the HCW were males (61.9%) and were doctors (62.4%). The mean age of participants was 32.8 years (SD 8.7) and majority were asymptomatic (51.8%). In this study, 33% of the HCW were reactive for SARS-CoV-2 IgG antibody. Around 44% of the reactive cases were asymptomatic. Factors which showed significant association with the presence of antibodies were professional category, type pf personal protective equipment available and living arrangement ($p<0.05$).

Conclusion:

Our study showed a high seropositivity of health care workers dealing with COVID-19 patients in Pakistan revealing significant association with professional category, nature of work place and precautions taken while performing duties.

Key words: Serosurveillance, Seropositivity, health care workers, COVID-19, SARS-CoV-2, IgG antibodies

Summary:

- Subclinical or asymptomatic cases can play a major role in SARS-CoV-2 transmission while remaining undiscovered in the community
- Health care workers (HCW) fall in the category of population which is most exposed to SARS-CoV-2 infection
- 33% of the health care workers were reactive for SARS-CoV-2 IgG antibody and 44% of the reactive cases were asymptomatic
- Our study revealed that there is a significant association between use of personal protective equipment, professional category, place of duty and seropositivity
- This study can help health care policy makers of Pakistan to know to extent of infection among the health care workers and make better strategies

Introduction:

COVID-19 (Corona Virus Disease 2019) associated with Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has emerged as a pandemic. Since the identification of initial cases of this atypical pneumonia, there has been an exponential increase in the number of cases worldwide.[1]Some of the few causes of this exponential increase in number include high transmission rate especially among the asymptomatic cases, poor health care strategies and limited knowledge about the novel corona virus.[2] Subclinical or asymptomatic cases are one of the major challenges in this pandemic as it is presumed that these cases can play a major role in infection spread while remaining undiscovered in the community.

As of August 22, 2020, confirmed cases of COVID-19 in Pakistan are 292,150 with 731 critical cases and 275,317 recoveries.[3] The correct estimate of the spread of COVID-19 in Pakistan is still not clear, posing a huge challenge to health care agencies of Pakistan. Most of the mildly symptomatic, asymptomatic cases and the cases belonging to areas of limited resources who are not tested via government health care agencies can easily be missed and the actual number of the affected individuals remains unidentified. The risk of COVID-19 among health care workers (HCW) of Pakistan is still not clear. Studies have revealed that 9% of the individuals in Italy and 26% of the individuals in Spain who were tested positive for COVID-19 via Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) in prevalence studies were health care workers. [4-5] In accordance with other coronaviruses, health care workers associated transmission of COVID 19 appears to play a major role in infection spread. One prevalence study suggested that more than 40% of the diagnosed cases of COVID 19 were assumed to have been acquired from health care workers.[6]

Majority of the SARS-CoV-2 infected cases remain asymptomatic and the virus may be far more widely distributed than experts may believe.[7] During the first 21 days of SARS-CoV-2 infection, reasonable increases are observed in virus specific IgM and IgG titers.[8] According to one estimate, herd immunity can be ensured if around 50 to 67% of the population develops immunity against SARS-CoV-2.[9] Seroconversion is determined by analyzing antibody status of the population. [9-10] Serosurveillance of HCW is an important indicator of COVID-19 spread as they fall in the category of population which is most exposed to SARS-CoV-2 infection.

In this study we aimed to find the seroprevalence of health care workers of Pakistan involved in the management and care of COVID-19 patients. This study will help to identify the high-risk subgroups of health care workers, high risk departments and different factors associated with the risk of SARS-CoV-2 infection.

Methodology:

Subject selection:

This was a cross sectional study approved by the Institutional Review Board of Chughtai Institute of Pathology, Lahore, Pakistan. A total of 15000 HCW, both male and females were randomly selected for this study from all over Pakistan. A list of health care centers who were authorized to treat and diagnose COVID-19 patients was issue by Government officials of Pakistan and only the HCW involved in providing services and care in these centers were involved in this study. All Doctors, Nurses/Para Medical Staff and

Medical Laboratory Technologists in Pakistan are registered in Pakistan Medical Council health registry, Pakistan Nursing Council and Medical Laboratory Technology Association of Pakistan respectively. The registration number of all health care professionals was confirmed from the authorities before including them in the study. The HCW with no interaction with COVID-19 patients (either direct contact or dealing with patient samples) or the workers who were not present on duty in the last one month from the date of commencement of study were excluded. Moreover, HCW who were already involved in some clinical trials of COVID-19 were not involved in the study. All participants were explained the objective of the study and the HCW showing active signs and symptoms compatible with COVID-19 were not involved in the study as they might be a risk to the team members of the laboratory taking samples and most probably be in the initial phase of the infection in which IgG is not likely to be present. After taking a written informed consent, the participants were asked to fill a questionnaire in which they had to mention their age, gender, professional category, place and nature of duty, kind of personal protective equipment provided to them, presence of sign and symptoms and living arrangement. A 3 ml random blood sample was collected from each participant to assess immunological response. Sample was collected by trained phlebotomist wearing appropriate personal protective equipment. Our laboratory centers are located throughout the country; therefore, our own designated sample collection team was sent to collect samples from all the participants. No local occupational health nurses were involved in sample collection. All the samples collected were transported immediately to the laboratory where those were centrifuged at 5000g for 5 min to separate sera for analysis.

Patient and public Involvement:

HCW involved in providing services and care to the COVID-19 patients were involved in the design and conduct of this study. Discussion sessions were arranged with a group of health care workers to discuss the feasibility of the project, designing proforma, outcome measures and sample collection procedure. This discussion group considered participants' priorities and preferences while designing the study. All the participants will be informed about the results of the study after publication through phone calls and lab reports posted if desired by the study participants

Laboratory analysis:

Analysis was done on serum samples for the qualitative detection of SARS-CoV-2 IgG antibodies using FDA (Food and Drug Administration) approved Abbott Chemiluminescent Microparticle Immunoassay on Abbott Alinity Ci. This is an EUA (Emergency Use Authorization) granted assay with a specificity of 99.6% and sensitivity of 96.7% for COVID-19 confirmed cases with more than 14 days of symptoms. An index of 1.4 was used as a cut off to mark reactive and non-reactive cases. Cases with results greater than the cut off value were marked as reactive and those with results less than the cut off were declared as non-reactive for SARS-CoV-2 IgG antibodies.

Statistical analysis:

SPSS version 23.0 was used for data analysis. Seroprevalence was calculated and associations between variables of the study were tested via Chi Square and Fisher's exact test using p value <0.05 as significant. Relative risk was expressed as odds ratio.

Results:

Baseline characteristics of the participants:

A total of 16882 HCW were randomly approached for the survey between 1st July 2020 to 20th July 2020. Of these, 1672 were excluded from the survey on the basis of exclusion criteria, 210 fulfilled the inclusion criteria but refused to participate and 15000 cases were selected with a total participation rate of 88.8%. These health care workers included doctors, nurses and paramedical staff directly involved in providing medical care to the COVID-19 patients. Out of all the cases, majority of the HCW were males (61.9%) and were doctors (62.4%) followed by paramedical staff (22%). The mean age of participants was 32.8 years ±8.7 and majority were asymptomatic (51.8%). The nature of personal protective equipment provided to health care workers, the place of duty, living arrangements along with some other characteristics of the participating HCW are mentioned in table 1.

Table 1: Baseline Characteristics of health care workers participating in the study

Groups	Subgroups	Total Number	Percentages
Gender	Male	9282	61.9
	Female	5718	38.1
Age (years)		Mean 32.8 ±8.7	
Category	Doctor	9362	62.4
	Nurse	2260	15.1
	Paramedics	3378	22.5
Place of duty	General Ward	6327	42.2
	OPD / Clinic	4010	26.7
	COVID-19 Quarantine center	291	1.9
	COVID-19 Isolation Ward	2820	18.8
	HDU / ICU	1445	9.6
	Emergency Unit	32	.2
	Laboratory	32	.2
	Operation Theater	43	.3
Tyvek Suits	Not Available	7784	51.9
	Available	7216	48.1
Face Shield	Not Available	9518	63.5
	Available	5482	36.5
Safety Goggles	Not Available	10299	68.7
	Available	4701	31.3
Gloves	Not Available	3849	25.7
	Available	11151	74.3
N95 Masks	Not Available	6514	43.4
	Available	8486	56.6
Surgical Masks	Not Available	4149	27.7
	Available	10851	72.3
Aymptomatic		7768	51.8

Reported COVID-19 compatible symptoms within last 2 month		6260	41.7
Experienced generalized fatigue only		972	6.5
Living arrangement	Living in hostel/Single Family	6089	40.5
	Living with family	8911	59.5

Seroprevalence of SRAS-CoV-2 IgG antibodies among the health care workers:

In this study, 33% of the health care workers were reactive for SARS-CoV-2 IgG antibody and maximum percentage of HCW (46%) showing seropositivity were between 23 to 30 years of age. Out of all the tests conducted, maximum number of participants were from Punjab (10,943) followed by Khyber Pakhtunkhwa (2606) (Figure 1) City wise seropositivity is given in Figure 2. Among the reactive cases, 40% of the HCW performed duties in general COVID-19 ward, 28 % were working in outpatient department receiving COVID-19 patients, 20% in COVID-19 isolation and quarantine centers and 10 % were working in COVID-19 high dependency units with patients having severe symptoms requiring mechanical respiratory support.

Around 2223 (44%) of the reactive cases were asymptomatic. The symptoms more significantly associated with seropositivity were; Low Grade fever only (OR 1.31 CI 1.16-1.48), High grade Fever with headache (OR 2.43 CI 2.16-2.73), Fever with cough and shortness of breath (OR 2.10 CI 1.91-2.31), Loss of sense of smell or taste only (OR 3.70 CI 3.29-4.17) ($p < 0.001$). Factors which showed significant association with the presence of antibodies were professional category, place of duty, availability of protective masks, safety goggles and living arrangements ($p < 0.05$). Relative risk of different variables expressed in terms of Odds ratio is given in table 2.

Table 2: Factor analysis of different variables associated with seropositivity

Groups	Subgroups	Non-Reactive	Reactive	P value	OR	(95% CI)
Gender	Male*	6117 (41%)	3165 (21%)	<0.001	0.87	0.81-0.94
	Female	3933 (26%)	1785 (12%)			
Category	Doctor*	6572 (44%)	2790 (19%)	<0.001	1.463	1.365-1.568
	Nurse	1409 (9%)	851 (6%)			
	Paramedics	2069	1309			

		(14%)	(8%)			
Place of duty	General Ward/Isolation Centers/Operation theater/ Lab*	9100 (61%)	4455 (30%)		1.064	0.949-1.193
	HDU / ICU	950 (6%)	495 (3%)	0.285		
Tyvek Suits	Not Available*	5184 (34%)	2600 (17%)	0.27	0.963	(0.89-1.03)
	Available	4866 (33%)	2350 (16%)			
Face Sheild	Not Available*	6331 (42%)	3190 (21%)	0.083	0.93	0.875-1.00
	Available	3719 (25%)	1760 (12%)			
Safety Goggles	Not Available*	6829 (45%)	3470 (24%)	0.008	.904	0.84-0.97
	Available	3221 (21%)	1480 (10%)			
Gloves	Not Available*	2592 (18%)	1257 (8%)	.601	1.021	0.944-1.104
	Available	7458 (50%)	3693 (24%)			
N95 Masks	Not Available*	4280 (29%)	2234 (15%)	.003	.902	0.842-0.966
	Available	5770 (38%)	2716 (18%)			
Surgical Masks	Not Available*	2812 (19%)	1337 (9%)	.212	1.050	0.973-1.133
	Available	7238 (48%)	3613 (24%)			
Aymptomatic*		5545 (37%)	2223 (15%)	<0.001	1.510	1.410-1.617
Reported COVID-19 compatible symptoms		4505 (30%)	2727 (18%)			

within last 2 month						
Living arrangement	Living alone/single family*	4173 (27%)	1916 (13%)	0.001	1.124	1.049-1.205
	Living with Joint family	5877 (39%)	3034 (21%)			

- OR= Odds ratio, CI= Confidence interval, * Reference group for odds ratio
- The reference group for the OR is the first mentioned group

Discussion:

Serosurveys are essential in the management of infectious diseases to assess the immunity in a population.[11] This is the first study reporting SRS-CoV-2 IgG antibodies seroprevalence among health care workers in Pakistan who are the frontline warriors in this pandemic. Pakistan is a developing nation with limited medical resources and health care workers are not provided with all the necessary equipment to protect themselves from this deadly virus. In this study we found a seropositivity of 33% among health care workers of Pakistan with highest seropositivity in the Punjab region of Pakistan with maximum disease burden.[3]The association of antibodies with virus neutralization, correlation of antibody titers with protection against re infection and difference in immunological response of symptomatic and asymptomatic cases is still unclear.[12] However the role of serological assays in assessing the prevalence of COVID-19 in the community is unquestionable.[13] We designed this study to investigate seroprevalence of SARS-CoV-2 IgG antibodies among the population involved in direct dealing with COVID-19 patients (either the patients or direct dealing with patient samples) with FDA approved assay having high analytical performance capable to identify IgG antibodies against N protein of the virus.[14] The HCW showing active signs and symptoms were excluded from the study to reduce the risk of exposure of laboratory staff collecting blood samples and also due to the probability of HCW being present in acute phase of the infection when IgG is not developed enough to be detected by the assay. Given the fact that HCW are at a very high risk of COVID-19, from our findings with a seropositivity of 33% among HCW it can be assumed that Pakistani population is still far from reaching more than 60% herd immunity level that is required for community protection against the infection. [15] Low odds ratio of some of the PPE (like protective masks) might be due to the fact that despite of existence, the PPE was not being used properly or they were not available in adequate quantity.

To date, many studies have been conducted worldwide to unfold prevalence of SARS-CoV-2 IgG antibodies among health care workers. Screening of symptomatic health care workers has been done with the help of RT-PCR in some surveillance studies and it was found that almost 18% of the HCW were infected by SARS-CoV-2.[16-17] Another screening study on asymptomatic HCW revealed that 3% of the staff was SARS-CoV-2 positive by RT-PCR .[18] In one study, the authors speculated that frontline health care workers are more prone to be infected by the virus. Not only the health professionals who are working in the dedicated COVID-19 health facilities are expected to be exposed but also the workers

who are performing duties in other specialties where SARS-CoV-2 patients are admitted for other reasons.[19] A study conducted on women admitted in labor room to give birth showed that 15% of the expecting mothers were SARS-CoV-2 positive and another study in old care home revealed 4% of the residents to be positive.[20-21] A recent seroprevalence study conducted in Denmark showed that 4% of the health care workers showed seropositivity against SARS-CoV-2 and the risk of viral infection was related to the level of exposure to the infected patients.[22] This study also revealed that seroconversion was higher in male health care workers as compared to females but the authors attributed this finding to higher frequency of critical illness in males rather than higher susceptibility of infection in male gender.[22] This finding is in accordance with our study in which male health care workers showed more frequent seroconversion as compared to females and seropositivity had an association with direct contact with the patients.

An Italian study found that 4.6% of the health care workers were seropositive, majority were men and low prevalence was found in asymptomatic cases.[23] All these findings are in accordance with our study which yielded similar findings. Another study conducted at a large Spanish hospital found a seroprevalence of 9.3% in a random sample of health care professionals and the authors concluded that HCW are at a higher risk to acquire SARS-CoV-2 infection from fellow workers or directly from the patients.[24] Our study revealed that there is a significant association between presence and absence of PPE and seropositivity. Some studies suggest that seropositivity is attributable to institutional application of proper PPE use hospitals which prepared well before time for this pandemic had a lower infection rate among their health care workers.[23] A special communication regarding risk assessment of health care workers performing duties at high risk areas suggested that lack of understanding of the disease course, improper use of PPE, stress at work, unavailability of screening tests and lack of resources are the major factors leading to high rate of infection among this cohort.[25] Our study has some limitations like only the antibody against the N protein was assessed, subjects were not followed up to understand the duration of seropositivity and protection from getting re infected again.

Conclusion:

This is a large-scale study conducted in all provinces of Pakistan showing a high seropositivity of health care workers dealing with COVID-19 patients. The seropositivity had significant association with place of duty, professional category and availability of PPE. This study can help health care policy makers of Pakistan to make better strategies to protect the front-line warriors in this pandemic.

FUNDING:
Chughtai Lab, Lahore, Pakistan.

ETHICAL APPROVAL:
Ethical approval was obtained by Institutional Review Board of Chughtai Institute of Pathology prior to initiation of the research work.

PATIENTS' CONSENT:
Informed consent was obtained from all patients to publish the data concerning this study. However, there is no chance that participants can be identified from the text or illustrations of this study.

CONFLICT OF INTEREST:
Authors declare no conflict of interest.

DECLARATION OF TRANSPARENCY:

Dr. Hijab Batool affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

Note: No additional data.

Figures:

Figure 1. Total number of tests conducted in each province of Pakistan and respective seropositivity

Figure 2. Seropositivity of health care workers in different cities of Pakistan

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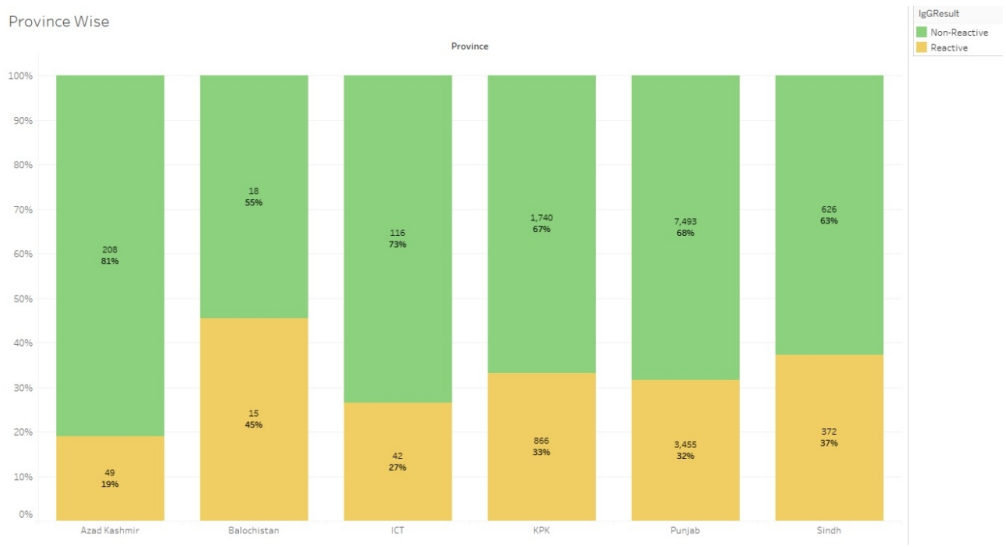

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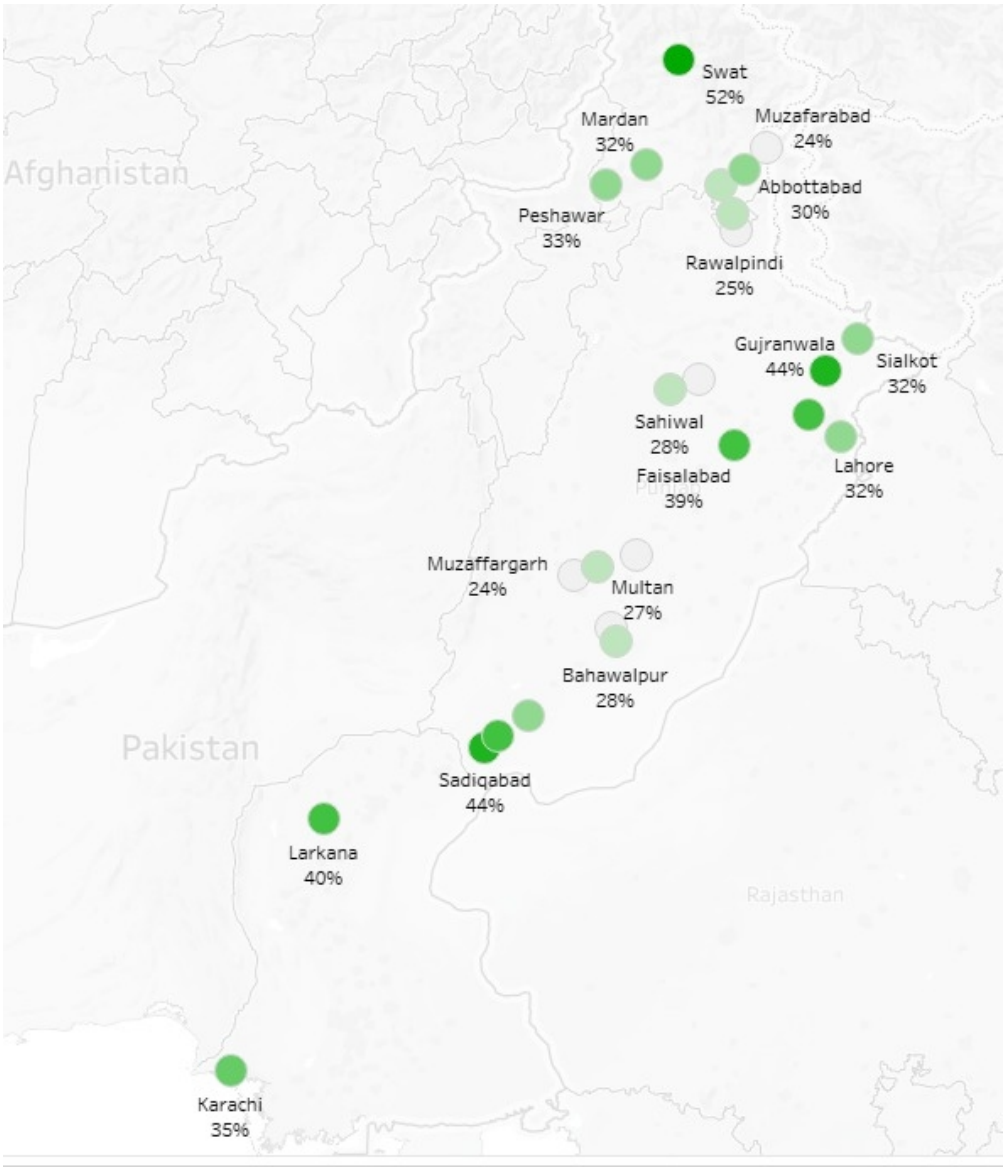

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STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2,4
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2,3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3,4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	2
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(*a*) *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	4
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*4*
Bias	9	Describe any efforts to address potential sources of bias	4
Study size	10	Explain how the study size was arrived at	4
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	4
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	4
		(*b*) Describe any methods used to examine subgroups and interactions	4
		(*c*) Explain how missing data were addressed	
		(*d*) *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	4
		(*e*) Describe any sensitivity analyses	N/A

Continued on next page

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	4
		(b) Give reasons for non-participation at each stage	4
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	5
		(b) Indicate number of participants with missing data for each variable of interest	N/A
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	N/A
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	*5*
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	5
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	18	Summarise key results with reference to study objectives	6
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	6
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	6
Generalisability	21	Discuss the generalisability (external validity) of the study results	6
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	7

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Seroprevalence of COVID-19 IgG Antibodies among Health Care Workers of Pakistan; A cross sectional study assessing exposure to COVID-19 and identification of high-risk subgroups

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Seroprevalence of COVID-19 IgG Antibodies among Health Care Workers of Pakistan; A cross sectional study assessing exposure to COVID-19 and identification of high-risk subgroups

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Contributor ship statement:

HB: Paper write-up, data collection and analysis, literature review.

ORC: Original concept, study design, supervision.

MDK: Proofreading, approval, discussion.

ASC: Proof reading and approval

SA: Data collection, statistical analysis.

JK: Data entry and analysis

Abstract:

Objective: In this study we aimed to find the seroprevalence of health care workers (HCW) of Pakistan involved in the treatment and care of COVID-19 patients.

Setting: This was a cross sectional study and total of 15000 HCW, involved in providing services and care to the COVID-19 patients were randomly selected from all over Pakistan.

Participants: Informed consent was taken from all participants and were included according to inclusion and exclusion criteria. All testing was done on serum samples for the qualitative detection of SARS-CoV-2 IgG antibodies using Abbott Chemiluminescent microparticle immunoassay. An index of 1.4 was used as a cut off to mark reactive and non-reactive cases. SPSS version 23.0 was used for data analysis.

Outcome: Immune status of the study population depicting seroprevalence among HCW

Results:

Out of all the candidates, majority of the HCW were males (61.9%) and were doctors (62.4%). The mean age of participants was 32.8 years (SD 8.7) and majority were asymptomatic (51.8%). In this study, 33% of the HCW were reactive for SARS-CoV-2 IgG antibody. Around 44% of the reactive cases were asymptomatic. The symptoms more significantly associated with seropositivity were; fever (OR 1.31 CI 1.16-1.48), headache (OR 2.43 CI 2.16-2.73), cough and shortness of breath (OR 2.10 CI 1.91-2.31), loss of sense of smell or taste (OR 3.70 CI 3.29-4.17) ($p < 0.001$). Factors which showed significant association with the presence of antibodies were professional category (AR 0.09 OR 1.46 CI 1.36-1.56), availability of protective masks (AR 0.02 OR 0.90 CI 0.84-0.96), safety goggles (AR 0.02 OR 0.90 CI 0.84-0.97) and living arrangements (AR 0.03 OR 1.12 CI 1.04-1.20) ($p < 0.05$).

Conclusion:

Our study showed a high seropositivity of health care workers dealing with COVID-19 patients in Pakistan revealing significant association with professional category, nature of work place and precautions taken while performing duties.

Key words: Serosurveillance, Seropositivity, health care workers, COVID-19, SARS-CoV-2, IgG antibodies

Strengths and limitations:

- First study in the country to report seroprevalence of COVID-IgG among health care workers
- Health policy makers can use the findings of our study to make better strategies
- High risk subgroups of health care workers and high-risk health departments were identified
- Only the antibody against the N protein of SARS-CoV-2 was assessed
- Subjects were not followed up to understand the duration of seropositivity

Summary:

- Subclinical or asymptomatic cases can play a major role in SARS-CoV-2 transmission while remaining undiscovered in the community
- Health care workers (HCW) fall in the category of population which is most exposed to SARS-CoV-2 infection
- 33% of the health care workers were reactive for SARS-CoV-2 IgG antibody and 44% of the reactive cases were asymptomatic
- Our study revealed that there is a significant association between use of personal protective equipment, professional category, place of duty and seropositivity
- This study can help health care policy makers of Pakistan to know to extent of infection among the health care workers and make better strategies

Introduction:

COVID-19 (Corona Virus Disease 2019) associated with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has emerged as a pandemic. Since the identification of initial cases of this atypical pneumonia, there has been an exponential increase in the number of cases worldwide.[1]Some of the few causes of this exponential increase in number include high transmission rate especially among the asymptomatic cases, poor health care strategies and limited knowledge about the novel corona virus.[2] Subclinical or asymptomatic cases are one of the major challenges in this pandemic as it is presumed that these cases can play a major role in infection spread while remaining undiscovered in the community.

As of August 22, 2020, confirmed cases of COVID-19 in Pakistan are 292,150 with 731 critical cases and 275,317 recoveries.[3] The correct estimate of the spread of COVID-19 in Pakistan is still not clear, posing a huge challenge to health care agencies of Pakistan. Most of the mildly symptomatic, asymptomatic cases and the cases belonging to areas of limited resources who are not tested via government health care agencies can easily be missed and the actual number of the affected individuals remains unidentified. The risk of COVID-19 among health care workers (HCW) of Pakistan is still not clear. Studies have revealed that 9% of the individuals in Italy and 26% of the individuals in Spain who were tested positive for COVID-19 via Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) in prevalence studies were health care workers. [4-5] In accordance with other coronaviruses, health care workers associated transmission of COVID 19 appears to play a major role in infection spread. One prevalence study suggested that more than 40% of the diagnosed cases of COVID 19 were assumed to have been acquired from health care workers.[6]

Majority of the SARS-CoV-2 infected cases remain asymptomatic and the virus may be far more widely distributed than experts may believe.[7] During the first 21 days of SARS-CoV-2 infection, reasonable increases are observed in virus specific IgM and IgG titers.[8] According to one estimate, herd immunity can be ensured if around 50 to 67% of the population develops immunity against SARS-CoV-2.[9]

Seroconversion is determined by analyzing antibody status of the population. [9-10] Serosurveillance of HCW is an important indicator of COVID-19 spread as they fall in the category of population which is most exposed to SARS-CoV-2 infection.

In this study we aimed to find the seroprevalence of health care workers of Pakistan involved in the management and care of COVID-19 patients. This study will help to identify the high-risk subgroups of health care workers, high risk departments and different factors associated with the risk of SARS-CoV-2 infection.

Methodology:

Subject selection:

This was a cross sectional study approved by the Institutional Review Board of Chughtai Institute of Pathology, Lahore, Pakistan. A total of 15000 HCW, both male and females were randomly selected for this study from all over Pakistan. A list of health care centers who were authorized to treat and diagnose COVID-19 patients was issue by Government officials of Pakistan and only the HCW involved in providing services and care in these centers were involved in this study. All Doctors, Nurses/Para Medical Staff and Medical Laboratory Technologists in Pakistan are registered in Pakistan Medical Council health registry, Pakistan Nursing Council and Medical Laboratory Technology Association of Pakistan respectively. The registration number of all health care professionals was confirmed from the authorities before including them in the study. The HCW with no interaction with COVID-19 patients (either direct contact or dealing with patient samples) or the workers who were not present on duty in the last one month from the date of commencement of study were excluded. Moreover, HCW who were already involved in some clinical trials of COVID-19 were not involved in the study. All participants were explained the objective of the study and the HCW showing active signs and symptoms compatible with COVID-19 were not involved in the study as they might be a risk to the team members of the laboratory taking samples and most probably be in the initial phase of the infection in which IgG is not likely to be present. After taking a written informed consent, the participants were asked to fill a questionnaire in which they had to mention their age, gender, professional category, place and nature of duty, kind of personal protective equipment provided to them, presence of sign and symptoms and living arrangement. A 3 ml random blood sample was collected from each participant to assess immunological response. Sample was collected by trained phlebotomist wearing appropriate personal protective equipment. Our laboratory centers are located throughout the country; therefore, our own designated sample collection team was sent to collect samples from all the participants. No local occupational health nurses were involved in sample collection. All the samples collected were transported immediately to the laboratory where those were centrifuged at 5000g for 5 min to separate sera for analysis.

Patient and public Involvement:

HCW involved in providing services and care to the COVID-19 patients were involved in the design and conduct of this study. Discussion sessions were arranged with a group of health care workers to discuss the feasibility of the project, designing proforma, outcome measures and sample collection procedure. This discussion group considered participants' priorities and preferences while designing the study. All the participants will be informed about the results of the study after publication through phone calls and lab reports posted if desired by the study participants

Laboratory analysis:

Analysis was done on serum samples for the qualitative detection of SARS-CoV-2 IgG antibodies using FDA (Food and Drug Administration) approved Abbott Chemiluminescent Microparticle Immunoassay on Abbott Alinity Ci. This is an EUA (Emergency Use Authorization) granted assay with a specificity of 99.6% and sensitivity of 96.7% for COVID-19 confirmed cases with more than 14 days of symptoms. An index of 1.4 was used as a cut off to mark reactive and non-reactive cases. Cases with results greater than the cut off value were marked as reactive and those with results less than the cut off were declared as non-reactive for SARS-CoV-2 IgG antibodies.

Statistical analysis:

SPSS version 23.0 was used for data analysis. Seroprevalence was calculated and associations between variables of the study were tested via Chi Square and Fisher's exact test using p value <0.05 as significant. Relative risk was expressed as odds ratio.

Results:

Baseline characteristics of the participants:

A total of 16882 HCW were randomly approached for the survey between 1st July 2020 to 20th July 2020. Of these, 1672 were excluded from the survey on the basis of exclusion criteria, 210 fulfilled the inclusion criteria but refused to participate and 15000 cases were selected with a total participation rate of 88.8%. These health care workers included doctors, nurses and paramedical staff directly involved in providing medical care to the COVID-19 patients. Out of all the cases, majority of the HCW were males (61.9%) and were doctors (62.4%) followed by paramedical staff (22%). The mean age of participants was 32.8 years ±8.7 and majority were asymptomatic (51.8%). The nature of personal protective equipment provided to health care workers, the place of duty, living arrangements along with some other characteristics of the participating HCW are mentioned in table 1.

Table 1: Baseline Characteristics of health care workers participating in the study

Groups	Subgroups	Total Number	Percentages
Gender	Male	9282	61.9
	Female	5718	38.1
Age (years)		Mean 32.8 ±8.7	
Category	Doctor	9362	62.4
	Nurse	2260	15.1
	Paramedics	3378	22.5
Place of duty	General Ward	6327	42.2
	OPD / Clinic	4010	26.7
	COVID-19 Quarantine center	291	1.9
	COVID-19 Isolation Ward	2820	18.8
	HDU / ICU	1445	9.6
	Emergency Unit	32	.2
	Laboratory	32	.2

	Operation Theater	43	.3
Tyvek Suits	Not Available	7784	51.9
	Available	7216	48.1
Face Shield	Not Available	9518	63.5
	Available	5482	36.5
Safety Goggles	Not Available	10299	68.7
	Available	4701	31.3
Gloves	Not Available	3849	25.7
	Available	11151	74.3
N95 Masks	Not Available	6514	43.4
	Available	8486	56.6
Surgical Masks	Not Available	4149	27.7
	Available	10851	72.3
Aymptomatic		7768	51.8
Reported COVID-19 compatible symptoms within last 2 month		6260	41.7
Experienced generalized fatigue only		972	6.5
Living arrangement	Living in hostel/Single Family	6089	40.5
	Living with family	8911	59.5

Seroprevalence of SRAS-CoV-2 IgG antibodies among the health care workers:

In this study, 33% of the health care workers were reactive for SARS-CoV-2 IgG antibody and maximum percentage of HCW (46%) showing seropositivity were between 23 to 30 years of age. Out of all the tests conducted, maximum number of participants were from Punjab (10,943) followed by Khyber Pakhtunkhwa (2606) (Figure 1) City wise seropositivity is given in Figure 2. Among the reactive cases, 40% of the HCW performed duties in general COVID-19 ward, 28 % were working in outpatient department receiving COVID-19 patients, 20% in COVID-19 isolation and quarantine centers and 10 % were working in COVID-19 high dependency units with patients having severe symptoms requiring mechanical respiratory support.

Around 2223 (44%) of the reactive cases were asymptomatic. The symptoms more significantly associated with seropositivity were; Low Grade fever only (OR 1.31 CI 1.16-1.48), High grade Fever with headache (OR 2.43 CI 2.16-2.73), Fever with cough and shortness of breath (OR 2.10 CI 1.91-2.31), Loss of sense of smell or taste only (OR 3.70 CI 3.29-4.17) ($p < 0.001$). Factors which showed significant association with the presence of antibodies were gender, professional category, availability of protective

masks, safety goggles and living arrangements (p <0.05). Absolute risk and Relative risk of different variables expressed in terms of Odds ratio is given in table 2.

Table 2: Factor analysis of different variables associated with seropositivity

Groups	Subgroups	Non-Reactive	Reactive	P value	OR	(95% CI)	AR	RR
Gender	Male*	6117 (41%)	3165 (21%)	<0.001	0.87	0.81-0.94	0.03	0.91
	Female	3933 (26%)	1785 (12%)					
Category	Doctor*	6572 (44%)	2790 (19%)	<0.001	1.463	1.365-1.568	0.09	1.31
	Nurse	1409 (9%)	851 (6%)					
	Paramedics	2069 (14%)	1309 (8%)					
Place of duty	General Ward/Isolation Centers/Operation theater/ Lab*	9100 (61%)	4455 (30%)	0.285	1.064	0.949-1.193	0.02	1.06
	HDU / ICU	950 (6%)	495 (3%)					
Tyvek Suits	Not Available*	5184 (34%)	2600 (17%)	0.27	0.963	(0.89-1.03)	0.01	0.96
	Available	4866 (33%)	2350 (16%)					
Face Sheild	Not Available*	6331 (42%)	3190 (21%)	0.083	0.93	0.875-1.00	0.01	0.93
	Available	3719 (25%)	1760 (12%)					
Safety Goggles	Not Available*	6829 (45%)	3470 (24%)	0.008	.904	0.84-0.97	0.02	0.93
	Available	3221 (21%)	1480 (10%)					
Gloves	Not Available*	2592 (18%)	1257 (8%)	.601	1.021	0.944-1.104	0.01	1.03
	Available	7458	3693					

		(50%)	(24%)					
N95 Masks	Not Available*	4280 (29%)	2234 (15%)	.003	.902	0.842-0.966	0.02	0.94
	Available	5770 (38%)	2716 (18%)					
Surgical Masks	Not Available*	2812 (19%)	1337 (9%)	.212	1.050	0.973-1.133	0.01	1.03
	Available	7238 (48%)	3613 (24%)					
Aymptomatic*		5545 (37%)	2223 (15%)	<0.001	1.510	1.410-1.617	0.09	1.32
Reported COVID-19 compatible symptoms within last 2 month		4505 (30%)	2727 (18%)					
Living arrangement	Living alone/single family*	4173 (27%)	1916 (13%)	0.001	1.124	1.049-1.205	0.03	1.09
	Living with Joint family	5877 (39%)	3034 (21%)					

- OR= Odds ratio, CI= Confidence interval, * Reference group for odds ratio, AR=Absolute Risk, RR= Relative risk
- The reference group for the OR is the first mentioned group

Discussion:

Serosurveys are essential in the management of infectious diseases to assess the immunity in a population.[11] This is the first study reporting SRS-CoV-2 IgG antibodies seroprevalence among health care workers in Pakistan who are the frontline warriors in this pandemic. Pakistan is a developing nation with limited medical resources and health care workers are not provided with all the necessary equipment to protect themselves from this deadly virus. In this study we found a seropositivity of 33% among health care workers of Pakistan with highest seropositivity in the Punjab region of Pakistan with maximum disease burden.[3]The association of antibodies with virus neutralization, correlation of antibody titers with protection against re infection and difference in immunological response of symptomatic and asymptomatic cases is still unclear.[12] However the role of serological assays in assessing the prevalence of COVID-19 in the community is unquestionable.[13] We designed this study to investigate seroprevalence of SARS-CoV-2 IgG antibodies among the population involved in direct dealing with COVID-19 patients (either the patients or direct dealing with patient samples) with FDA

approved assay having high analytical performance capable to identify IgG antibodies against N protein of the virus.[14] The HCW showing active signs and symptoms were excluded from the study to reduce the risk of exposure of laboratory staff collecting blood samples and also due to the probability of HCW being present in acute phase of the infection when IgG is not developed enough to be detected by the assay. Given the fact that HCW are at a very high risk of COVID-19, from our findings with a seropositivity of 33% among HCW it can be assumed that Pakistani population is still far from reaching more than 60% herd immunity level that is required for community protection against the infection. [15] Low odds ratio of some of the PPE (like protective masks) might be due to the fact that despite of existence, the PPE was not being used properly or they were not available in adequate quantity.

To date, many studies have been conducted worldwide to unfold prevalence of SARS-CoV-2 IgG antibodies among health care workers. Screening of symptomatic health care workers has been done with the help of RT-PCR in some surveillance studies and it was found that almost 18% of the HCW were infected by SARS-CoV-2.[16-17] Another screening study on asymptomatic HCW revealed that 3% of the staff was SARS-CoV-2 positive by RT-PCR .[18] In one study, the authors speculated that frontline health care workers are more prone to be infected by the virus. Not only the health professionals who are working in the dedicated COVID-19 health facilities are expected to be exposed but also the workers who are performing duties in other specialties where SARS-CoV-2 patients are admitted for other reasons.[19] A study conducted on women admitted in labor room to give birth showed that 15% of the expecting mothers were SARS-CoV-2 positive and another study in old care home revealed 4% of the residents to be positive.[20-21] A recent seroprevalence study conducted in Denmark showed that 4% of the health care workers showed seropositivity against SARS-CoV-2 and the risk of viral infection was related to the level of exposure to the infected patients.[22] This study also revealed that seroconversion was higher in male health care workers as compared to females but the authors attributed this finding to higher frequency of critical illness in males rather than higher susceptibility of infection in male gender.[22] This finding is in accordance with our study in which male health care workers showed more frequent seroconversion as compared to females and seropositivity had an association with direct contact with the patients.

An Italian study found that 4.6% of the health care workers were seropositive, majority were men and low prevalence was found in asymptomatic cases.[23] All these findings are in accordance with our study which yielded similar findings. Another study conducted at a large Spanish hospital found a seroprevalence of 9.3% in a random sample of health care professionals and the authors concluded that HCW are at a higher risk to acquire SARS-CoV-2 infection from fellow workers or directly from the patients.[24] Our study revealed that there is a significant association between presence and absence of PPE and seropositivity. Some studies suggest that seropositivity is attributable to institutional application of proper PPE use hospitals which prepared well before time for this pandemic had a lower infection rate among their health care workers.[23] A special communication regarding risk assessment of health care workers performing duties at high risk areas suggested that lack of understanding of the disease course, improper use of PPE, stress at work, unavailability of screening tests and lack of resources are the major factors leading to high rate of infection among this cohort.[25] Our study has some limitations like only the antibody against the N protein was assessed, subjects were not followed up to understand the duration of seropositivity and protection from getting re infected again.

Conclusion:

This is a large-scale study conducted in all provinces of Pakistan showing a high seropositivity of health care workers dealing with COVID-19 patients. The seropositivity had significant association with place of duty, professional category and availability of PPE. This study can help health care policy makers of Pakistan to make better strategies to protect the front-line warriors in this pandemic.

FUNDING:
Chughtai Lab, Lahore, Pakistan.

ETHICAL APPROVAL:
Ethical approval was obtained by Institutional Review Board of Chughtai Institute of Pathology prior to initiation of the research work.

PATIENTS' CONSENT:
Informed consent was obtained from all patients to publish the data concerning this study. However, there is no chance that participants can be identified from the text or illustrations of this study.

CONFLICT OF INTEREST:
Authors declare no conflict of interest.

DECLARATION OF TRANSPARENCY:

Dr. Hijab Batool affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

Note: No additional data.

Figures:

Figure 1. Total number of tests conducted in each province of Pakistan and respective seropositivity

Figure 2. Seropositivity of health care workers in different cities of Pakistan

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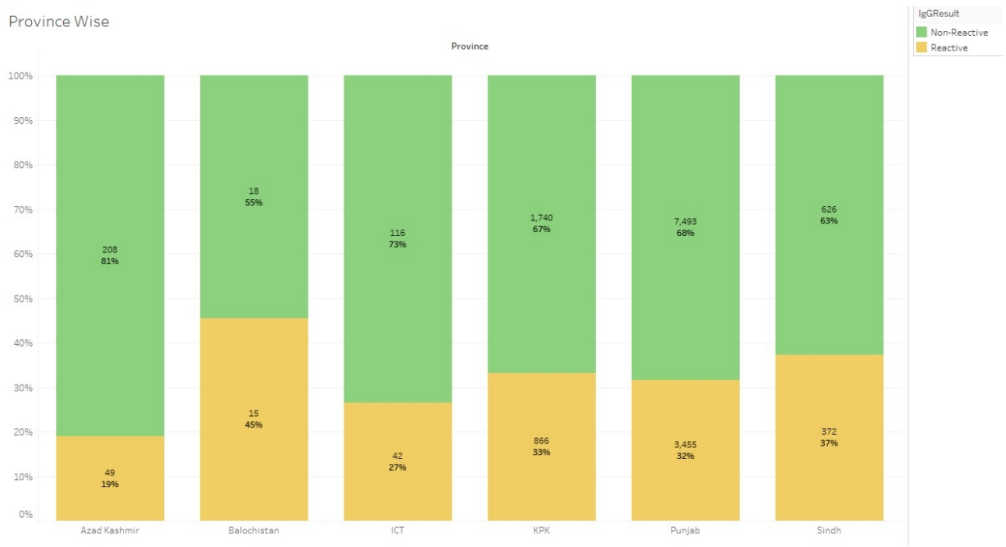

Figure 1. Total number of tests conducted in each province of Pakistan and respective seropositivity

451x243mm (72 x 72 DPI)

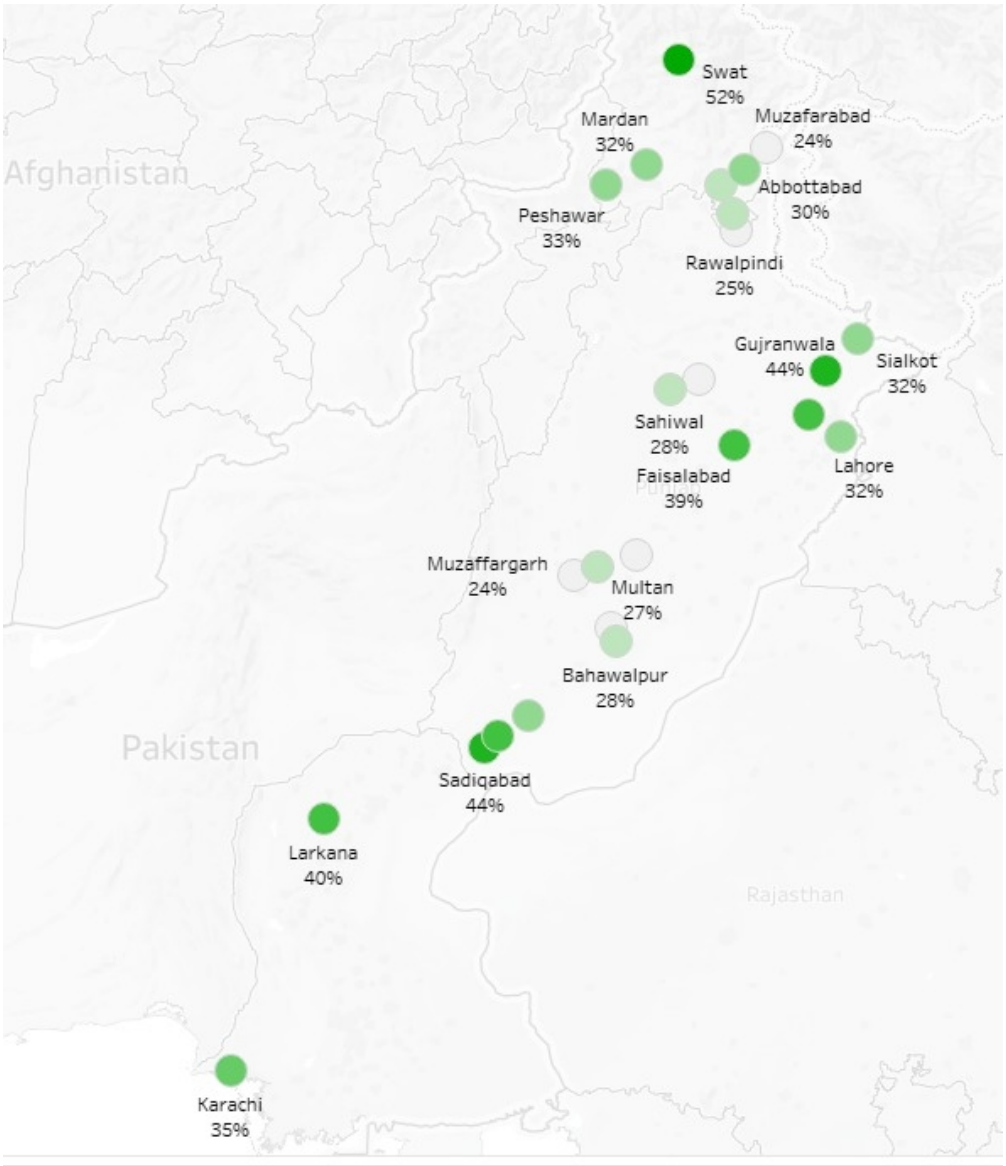

Figure 2. Seropositivity of health care workers in different cities of Pakistan

208x242mm (72 x 72 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2,4
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2,3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3,4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	2
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(*a*) *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	4
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*4*
Bias	9	Describe any efforts to address potential sources of bias	4
Study size	10	Explain how the study size was arrived at	4
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	4
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	4
		(*b*) Describe any methods used to examine subgroups and interactions	4
		(*c*) Explain how missing data were addressed	N/A
		(*d*) *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	4
		(*e*) Describe any sensitivity analyses	N/A

Continued on next page

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	4
		(b) Give reasons for non-participation at each stage	4
		(c) Consider use of a flow diagram	N/A
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	5
		(b) Indicate number of participants with missing data for each variable of interest	N/A
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	N/A
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	*N/A*
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	*N/A*
		Cross-sectional study—Report numbers of outcome events or summary measures	*5*
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	5
		(*b*) Report category boundaries when continuous variables were categorized	N/A
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	18	Summarise key results with reference to study objectives	6
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	6
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	6
Generalisability	21	Discuss the generalisability (external validity) of the study results	6
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	7

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.